WHAT IS CLAIMED IS:

Sub Q1

1. A vector construct comprising:

(a) a first transcriptional regulatory sequence operably linked to a first unpaired splice donor sequence;

(b) a second transcriptional regulatory sequence operably linked to a second unpaired splice donor sequence; and

(c) a linearization site.

2. The vector construct of claim 1, wherein said linearization site is located between said first unpaired splice donor site and said second transcriptional regulatory sequence.

3. The vector construct of claim 1, wherein when said vector integrates into the genome of a host cell, said first transcriptional regulatory sequence is in an inverted orientation relative to the orientation of said second transcriptional regulatory sequence.

4. The vector of claim 1, wherein said vector has been rendered linear by cleavage at said linearization site.

5. A vector construct comprising, in sequential order:

(a) a transcriptional regulatory sequence;

(b) an unpaired splice donor site;

(c) a rare cutting restriction site; and

(d) a linearization site.

Sub Q2

6. A vector construct comprising, in sequential order:

(a) a transcriptional regulatory sequence;

(b) a vector-encoded exon comprising a rare cutting restriction site;

(c) an unpaired splice-donor site; and

(d) a linearization site.

Sub A2

7. A vector construct comprising, in sequential order:

(a) a transcriptional regulatory sequence;

(b) a vector-encoded exon comprising a first rare cutting restriction site;

(c) an unpaired splice-donor site;

(d) a second rare cutting restriction site; and

(e) a linearization site.

8. A vector construct comprising:

(a) a first transcriptional regulatory sequence operably linked to a selectable marker lacking a polyadenylation signal; and

(b) a second transcriptional regulatory sequence operably linked to an exon-splice donor site complex,

wherein said first transcriptional regulatory sequence is in the same orientation in said vector construct as said second transcriptional regulatory sequence.

9. A vector construct comprising a transcriptional regulatory sequence operably linked to a selectable marker lacking a polyadenylation signal, and further comprising an unpaired splice donor site.

10. A vector construct comprising a first transcriptional regulatory sequence operably linked to a selectable marker lacking a polyadenylation signal, and further comprising a second transcriptional regulatory sequence operably linked to an unpaired splice donor site.

Sub A3

11. The vector construct of any one of claims 1, 8, or 10, wherein said first transcriptional regulatory sequence or said second transcriptional regulatory sequence is a promoter.

12. The vector construct of claim 11, wherein said promoter is selected from the group consisting of a CMV immediate early gene promoter, an SV40 T antigen promoter, a tetracycline-inducible promoter, and a β-actin promoter.

13. The vector construct of any one of claims 5-7 or 9, wherein said transcriptional regulatory sequence is a promoter.

14. The vector construct of claim 13, wherein said promoter is selected from the group consisting of a CMV immediate early gene promoter, an SV40 T antigen promoter, a tetracycline-inducible promoter, and a β-actin promoter.

15. The vector construct of any one of claims 8-10, wherein said selectable marker is selected from the group consisting of a neomycin gene, a hypoxanthine phosphribosyl transferase gene, a puromycin gene, a dihydrooratase gene, a glutamine synthetase gene, a histidine D gene, a carbamyl phosphate synthase gene, a dihydrofolate reductase gene, a multidrug resistance 1 gene, an aspartate transcarbamylase gene, a xanthine-guanine phosphoribosyl transferase gene, an adenosine deaminase gene, and a thymidine kinase gene.

16. A vector construct comprising:

(a) a positive selectable marker;

(b) a negative selectable marker; and

(c) an unpaired splice donor site,

wherein said positive and negative selectable markers and said splice donor site are oriented in said vector construct in an orientation that results in expression of said positive selectable marker in active form, and either non-expression of said negative selectable marker or expression of said negative selectable marker in inactive form, when said vector construct is integrated into the genome of a eukaryotic host cell in such a way that an endogenous gene in said genome is activated.

17. The vector construct of claim 16, wherein said positive selection marker and said negative selection marker both lack a polyadenylation signal.

18. The vector construct of claim 16, wherein said positive selection marker is selected from the group consisting of a neomycin gene, a hypoxanthine phosphribosyl transferase gene, a puromycin gene, a dihydrooratase gene, a glutamine synthetase gene, a histidine D gene, a carbamyl phosphate synthase gene, a dihydrofolate reductase gene, a multidrug resistance 1 gene, an aspartate transcarbamylase gene, a xanthine-guanine phosphoribosyl transferase gene, and an adenosine deaminase gene.

19. The vector construct of claim 16, wherein said negative selection marker is selected from the group consisting of a hypoxanthine phosphribosyl transferase gene, a thymidine kinase gene, and a diphtheria toxin gene.

20. A eukaryotic host cell comprising the vector construct of any one of claims 1, 5-10, or 16.

21. The eukaryotic host cell of claim 20, wherein said cell is an animal cell.

22. The eukaryotic host cell of claim 21, wherein said animal cell is selected from the group consisting of a mammalian cell, an insect cell, an avian cell, an annelid cell, an amphibian cell, a reptilian cell, and a fish cell.

23. The eukaryotic host cell of claim 21, wherein said animal cell is a mammalian cell.

24. The eukaryotic host cell of claim 23, wherein said mammalian cell is a human cell.

25. The eukaryotic host cell of claim 20, wherein said cell is a plant cell.

26. The eukaryotic host cell of claim 20, wherein said cell is a fungal cell.

27. The eukaryotic host cell of claim 26, wherein said fungal cell is a yeast cell.

28. The eukaryotic host cell of claim 21, wherein said cell is an isolated cell.

29. The eukaryotic host cell of claim 21, wherein said vector construct is integrated into the genome of said host cell.

30. A primer molecule comprising a PCR-amplifiable sequence and a degenerate 3' terminus, wherein said primer molecule has the structure:

$$5'\text{-}(dT)_a\text{-}X\text{-}N_b\text{-}TTTATT\text{-}3',$$

wherein a is a whole number from 1 to 100, X is a PCR-amplifiable sequence consisting of a nucleic acid sequence of about 10-20 nucleotides in length, N is any nucleotide, and b is a whole number from 0 to 6.

31. The primer molecule of claim 30, wherein said PCR-amplifiable sequence comprises one or more restriction sites.

32. The primer molecule of claim 30, wherein a is a whole number from 10 to 30.

33. The primer molecule of claim 30, wherein said primer molecule comprises one or more hapten molecules conjugated to one or more bases of said primer molecule.

34. The primer molecule of claim 33, wherein said hapten molecules are selected from the group consisting of biotin, digoxigenin, an antibody, an enzyme, lipopolysaccharide, apotransferrin, ferrotransferrin, insulin, a cytokine an extracellular matrix protein, an integrin, ankyrin, C3bi, fibrinogen, spectrin, a cytokine receptor, an insulin receptor, a transferrin receptor, polymyxin B, endotoxin-neutralizing protein (ENP), an enzyme-specific substrate, protein A, protein G, a cell-surface Fc receptor, an antibody-specific antigen, an antibody-specific peptide, avidin, and streptavidin.

35. The primer molecule of claim 33, wherein said hapten molecule is biotin.

36. A method for first strand cDNA synthesis comprising:

(a) annealing a primer of claim 30 to an RNA template molecule to form an primer-RNA complex; and

(b) treating said primer-RNA complex with reverse transcriptase and one or more deoxynucleoside molecules under conditions favoring the reverse transcription of said primer-RNA complex to synthesize a first strand cDNA.

37. A method for isolating an activated gene from a host cell genome, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence, a vector-encoded exon, an unpaired splice donor site, and a vector-encoded intron into a host cell;

(b) allowing said vector to integrate into the genome of said host cell by non-homologous recombination, under conditions such that said vector activates an endogenous gene in said genome;

(c) isolating RNA from said host cell;

(d) synthesizing first strand cDNA by reverse transcription of said isolated RNA;

(e) annealing a primer specific for said vector-encoded exon to said first strand cDNA to create a primer-first strand cDNA complex; and

(f) contacting said primer-first strand cDNA complex with a DNA polymerase under conditions favoring the production of a second strand cDNA product substantially complementary to said first strand cDNA.

38. A method for isolating an activated gene from a host cell genome, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence, a vector-encoded exon, an unpaired splice donor site, and a vector-encoded intron into a plurality of host cells;

(b) allowing said vector to integrate into the genomes of said host cells by non-homologous recombination, under conditions such that said vector activates an endogenous gene in said genomes;

(c) cultivating said host cells under conditions favoring the production of a plurality of individual clones from said host cells, wherein each of said individual clones in said plurality of clones contains said vector integrated into a unique site in said host cell genome;

(d) isolating RNA from said plurality of clones;

(e) synthesizing first strand cDNA by reverse transcription of said isolated RNA;

(f) annealing a first primer specific for said vector-encoded exon to said first strand cDNA to create a primer-first strand cDNA complex; and

(g) contacting said primer-first strand cDNA complex with a DNA polymerase under conditions favoring the production of a second strand cDNA product substantially complementary to said first strand cDNA.

39. The method of claim 37, further comprising treating said second strand cDNA product with a restriction enzyme that cleaves at a restriction site located on said vector-encoded exon.

40. The method of claim 38, further comprising treating said second strand cDNA product with a restriction enzyme that cleaves at a restriction site located on said vector-encoded exon.

41. The method of claim 37, further comprising treating said second strand cDNA product with a restriction enzyme that cleaves at a restriction site located on said vector-encoded intron downstream of said unpaired splice donor site.

42. The method of claim 38, further comprising treating said second strand cDNA product with a restriction enzyme that cleaves at a restriction site located on said vector-encoded intron downstream of said unpaired splice donor site.

43. The method of claim 37, further comprising amplifying said second strand cDNA product using a second primer specific for said vector-encoded exon and a third primer specific for said first primer.

44. The method of claim 38, further comprising amplifying said second strand cDNA product using a second primer specific for said vector-encoded exon and a third primer specific for said first primer.

45. An isolated gene produced according to the method of any one of claims 37-44.

46. A host cell comprising the isolated gene of claim 45.

47. A vector comprising the isolated gene of claim 45.

48. The vector of claim 47, wherein said vector is an expression vector.

49. A method of producing a polypeptide, comprising:

(a) introducing the vector of claim 47 into a host cell; and

(b) culturing said host cell under conditions favoring the expression by said host cell of a polypeptide encoded by said isolated gene.

50. The method of claim 49, further comprising isolating said polypeptide.

51. A polypeptide produced according to the method of claim 49 or claim 50.

52. A method of producing a polypeptide, comprising:

(a) introducing into a host cell a vector comprising a transcriptional regulatory sequence operably linked to an exonic region followed by an unpaired splice donor site, under conditions favoring the integration of said vector into the genome of said host cell and resulting in the activation of an endogenous gene in said genome; and

(b) culturing said host cell under conditions favoring the expression by said host cell of a polypeptide at least partially encoded by said exonic region,

wherein said exon contains a translational start site positioned at position -3, or at an increment of 3 bases upstream therefrom, from the 5'-most base of said splice donor site.

53. A method of producing a polypeptide, comprising:

(a) introducing into a host cell a vector comprising a transcriptional regulatory sequence operably linked to an exonic region followed by an unpaired splice donor site, under conditions favoring the integration of said vector into the genome of said host cell and resulting in the activation of an endogenous gene in said genome; and

(b) culturing said host cell under conditions favoring the expression by said host cell of a polypeptide at least partially encoded by said exonic region,

wherein said exon contains a translational start site positioned at position -2, or at an increment of 3 bases upstream therefrom, from the 5'-most base of said splice donor site.

54. A method of producing a polypeptide, comprising:

(a) introducing into a host cell a vector comprising a transcriptional regulatory sequence operably linked to an exonic region followed by an unpaired splice donor site, under conditions favoring the integration of said vector into the genome of said host cell and resulting in the activation of an endogenous gene in said genome; and

(b) culturing said host cell under conditions favoring the expression by said host cell of a polypeptide at least partially encoded by said exonic region,

wherein said exon contains a translational start site positioned at position -1, or at an increment of 3 bases upstream therefrom, from the 5'-most base of said splice donor site.

55. The method of any one of claims 52-54, further comprising isolating said polypeptide.

56. A polypeptide produced by any one of claims 52-54.

57. A polypeptide produced by the method of claim 55.